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Temperature and Population Density Effects on Locomotor Activity of *Musca domestica* (Diptera: Muscidae)

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ABSTRACT The behavior of ectotherm organisms is affected by both abiotic and biotic factors. However, a limited number of studies have investigated the synergistic effects on behavioral traits. This study examined the effect of temperature and density on locomotor activity of *Musca domestica* (L.). Locomotor activity was measured for both sexes and at four densities (with mixed sexes) during a full light and dark (L:D) cycle at temperatures ranging from 10 to 40°C. Locomotor activity during daytime increased with temperature at all densities until reaching 30°C and then decreased. High-density treatments significantly reduced the locomotor activity per fly, except at 15°C. For both sexes, daytime activity also increased with temperature until reaching 30 and 35°C for males and females, respectively, and thereafter decreased. Furthermore, males showed a significantly higher and more predictable locomotor activity than females. During nighttime, locomotor activity was considerably lower for all treatments. Altogether the results of the current study show that there is a significant interaction of temperature and density on daytime locomotor activity of *M. domestica* and that houseflies are likely to show significant changes in locomotor activity with change in temperature.

KEY WORDS flight, density, transmission, circadian rhythm, vector

Locomotion, growth, and reproduction of ectotherm organisms are highly affected by the ambient temperature (Deutsch et al. 2008). Thus, changes in temperature can be of great importance for a population's persistence (Randall et al. 2002, Podrabsky and Somero 2004). Organisms have therefore evolved sophisticated behavioral, physiological, and biochemical mechanisms to cope with temperature (Hoffmann and Parsons 1991). Many studies on insects have focused on physiological and biochemical adaptations to temperature, but behavior and the underlying physiological mechanisms are also likely to change with temperature (Hoffmann and Parsons 1991, Angilletta 2009). Behavioral responses can have important implications for an organism's ability to respond to changing temperatures (Kearney et al. 2009).

Temperature changes are as a result likely to affect the abundance and distribution of plants and animals and have therefore received considerable attention (Walther et al. 2002, Thomas et al. 2004, Thuiller et al. 2005, Wilson et al. 2005). In a number of species, range shifts have already been documented toward the poles as a result of climate warming (Parmesan and Yohe 2003, Umina et al. 2005) and further range shifts are predicted to occur (Roura–Pascual et al. 2004, Goulson et al. 2005). Studies have also shown that several species increase the annual activity period and emerge earlier in the spring (Walther et al. 2002).

Much theoretical and empirical work has focused on the likely effects of climate warming on disease vectors (Martens et al. 1999, Githeko et al. 2000, Kovats et al. 2001). It has been found that vector-borne pathogens are particularly sensitive to climate warming (Rogers and Randolph 2003). In some instances, studies have shown a strong association between climate warming and increased risk of disease dispersal and transmission because of change in vector range, as found with the emergence of bluetongue disease in Europe (Purse et al. 2005). It has been suggested that other vector pathogens sharing some characteristics of the bluetongue virus (fast evolving, promiscuous agents that reproduce rapidly and are highly mobile habitat-generalist vectors) might also respond rapidly to increased climatic suitability (Purse et al. 2005).

Another factor that greatly affects many aspects of insects is density, although, as pointed out, this trait is complex and under natural condition interacts with a highly variable environment including temperature (Peters and Barbosa 1977). In *Musca domestica* (L.), density has been found to affect traits such as life expectancy and wing retention (Rockstein et al. 1981). Some studies have addressed the effect of density per se on behavioral traits, such as locomotor

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activity (Sewell 1979, Bahrndorff et al. 2012), although only under one set of thermal conditions and for either males or females. In addition, studies have found that larval density in *Drosophila* can also affect thermal performance, where larvae reared at high densities show increased expression of heat shock proteins and increased thermal performance (Sørensen and Loeschcke 2001).

M. domestica is a synanthropic species living in close association with humans and is a widespread species with large latitudinal ranges (Crosskey and Lane 1993). The larvae develop in decomposing organic material such as in fecal sources, whereas adults may feed on human food, domestic animal food, refuse, and excrement interchangeably (Crosskey and Lane 1993), making the adults important carriers of agents that can cause human and domestic animal diseases (Levine and Levine 1991). Studies have found a close link between environmental conditions and population dynamics of *M. domestica* (Goulson et al. 2005), and it has been shown that flies are able to reproduce rapidly, with large fluctuations in population density changing by up to two orders of magnitude within a few days (Goulson et al. 1999b, Pie et al. 2004).

Although studies have provided some evidence for the importance of temperature and density on the behavior of *M. domestica* and other insects (Sewell 1979, Buchan and Sohal 1981, Rockstein et al. 1981, Bahrndorff et al. 2012), this support is almost exclusively based on measurements at a limited number of temperatures or densities. However, an understanding of how temperature and density interact may help us unravel complex interactions and understand how it affects behavioral traits. In this study, we tested how locomotor activity of *M. domestica* was affected by different temperature and density treatments by using a full factorial design and by analyzing locomotor activity and its predictability.

Materials and Methods

Insect Rearing. Houseflies were obtained from a Danish dairy cattle farm in 1989 and since reared continuously under laboratory conditions at high population size (2–3,000) at 25°C and 80% relative humidity (RH). For this study, newly laid eggs were transferred to a medium consisting of wheat bran (24.6%), alfalfa (12.3%), yeast (0.6%), malt (0.9%), and tap water (61.6%). The medium was stirred gently once a day to increase gas and heat exchange. As the larvae pupated, the pupae were separated from the medium individually, by using a pair of forceps, and placed in a 200-ml vial sealed with a foam stopper allowing gas exchange and with 40 flies in each vial. Water was supplied in 1.5-ml Eppendorf tubes with cotton wool stoppers and food was supplied in form of sugar cubes. Larvae, pupae, and adult flies were all held in temperature cabinets at 25°C with a photoperiod of 16:8 (L:D) h.

Experimental Setup. Locomotor activity was measured by using a Locomotor Activity Monitor (LAM) (TriKinetics Inc., Waltham, MA). This was done by transferring flies to vials (6.5-cm-long capillary glass with an external diameter of 2.5 cm) and positioned horizontally in the LAM. Each end of the vials was sealed with foam stoppers and with food placed in the opposite end of the light source (Bahrndorff et al. 2012). The flies were sexed (briefly anesthetized by use of CO_2) and then transferred to the glass vials and left for 22 h before locomotor activity was measured. The activity within a vial was estimated as the number of times in a 5-s interval that a photocell in the middle of the vial was crossed. Light was provided by using an LED light source giving 70 lux. Parchment paper in front of the vials was used to ensure equal light intensity for all vials. Flies at an age of 48 ± 24 h were used in all the experiments.

Thermal Performance. The LAM with the vials containing the flies was placed in a temperature cabinet (Binder, Tuttlingen, Germany) to establish performance at the different temperatures. The vials were randomly assigned in the LAM and placed in a temperature cabinet at a given temperature. The first 1.5 h that followed setting up the flies in the morning in the temperature cabinet was dark, where the flies acclimated to the new environment, followed by 16 h of light and 6 h of dark before the experiment was stopped. Locomotor activity was recorded at the following temperatures: 10 ± 1, 15 ± 1, 20 ± 1, 25 ± 1, 30 ± 1, 35 ± 1, and 40 ± 1°C. For each temperature examined, five replicates of each sex (five males or five females kept in separate vials) and four density treatments (one pair, two pairs, three pairs, or four pairs) were examined. Each pair consisted of one male and one female. In total, this gave six treatment groups, with 30 vials at each temperature tested.

Statistical Analyses. The activity registered in each vial was divided into activity recorded during the light or dark period. The first 1.5 h in dark and also the first half hour before and after light shift were excluded to avoid activity measurements during abrupt and unnatural light shifts. This gave a daytime period of 15 h and a nighttime period of 5 h for analysis. These periods are referred to as daytime activity and nighttime activity.

To test for differences in activity between treatments, the activity registered in each replicate was summed. Subsequently, activity was divided by number of flies or pairs in the vial. By doing so, the measure of activity was either activity per fly for males and females or activity per pair for the different density treatments during either daytime or nighttime. We analyzed the data for differences in level of activity as well as predictability of the activity (autocorrelation time).

The data were analyzed by using a GLM framework in R V.2.11.1. (Development Core Team 2010). In our case, we assumed the activity and autocorrelation time to follow the quasi-Poisson distribution. This distribution is an extension of a Poisson distribution, which allows the variance to be different from, although still proportional to, the mean and does not require variance homogeneity between the samples. It should be noted that following the model, the predicted variance of the total activity of two flies divided by two is slightly different from the predicted activity of a single

fly even if the flies are independent. However, the effect of this is negligible, and more complex analyses on total activity instead of analyses of per individual activity were found to produce virtually identical results (results not shown). The data were also analyzed by using log transformation, and the overall patterns were the same as when by using raw data.

In all cases, the daytime and nighttime parameters were analyzed separately. First we analyzed all temperatures combined, while keeping time periods separate to understand how the effect of density interacts with the effect of temperatures. The initial model had five parameters incorporating linear and nonlinear effects of temperature or density (temperature, temperature^2, temperaure^3, density, and density^2) and the six first-order interactions between the three temperature parameters and the two density parameters. The *P* value of each parameter was calculated by using Wald's tests, and parameters were removed until only significant effects remained. Parameters were, however, kept in the model if any of their interactions were significant or if higher-order effects were significant. For daytime and nighttime activity, we also performed individual analyses at each temperature estimating the effect of density on activity. In these analyses, a negative slope will indicate that the average activity per pair is lower for higher densities, whereas a positive slope will indicate higher average activity per pair with increase in density. If the slope is 0, density has no effect on activity. We did not perform this analysis on autocorrelation time because our results showed that autocorrelation time was maximized at intermediate density, making this analysis inappropriate.

The effect of sex was analyzed by using similar analyses. In all cases, sex was treated as a dummy variable. First, the combined data from all temperatures were analyzed to understand the interaction between sex and temperature. The initial model in the combined analysis had four main parameters (temperature, temperature^2, temperaure^3, and sex) and the three interactions between sex and the linear or nonlinear effect of temperature. Individual analyses at each temperature were performed to analyze the difference between males and females in daytime activity and autocorrelation time. No individual analysis was performed on nighttime activity because the analysis of all temperatures combined did not find any overall patterns.

To estimate the strength of autocorrelation, we calculated the minimum time period (T) for each vial where the autocorrelation between the activity of a vial at time x and at time x + T was negative during the daytime period by using R V.2.11.1. (Development Core Team 2010). This will be referred to as autocorrelation time. We did not calculate the autocorrelation time in the nighttime period because flies in many vials did not have any activity. Autocorrelation cannot be defined in a series only containing zeros, and removal of all vials without activity would produce a biased subset. A single vial at daytime (one of the replicates with three pairs of flies) at 10°C also had no activity and was excluded from analyses of autocorrelation, but because only one vial of 140 was removed, we consider it unlikely that it will influence our results.

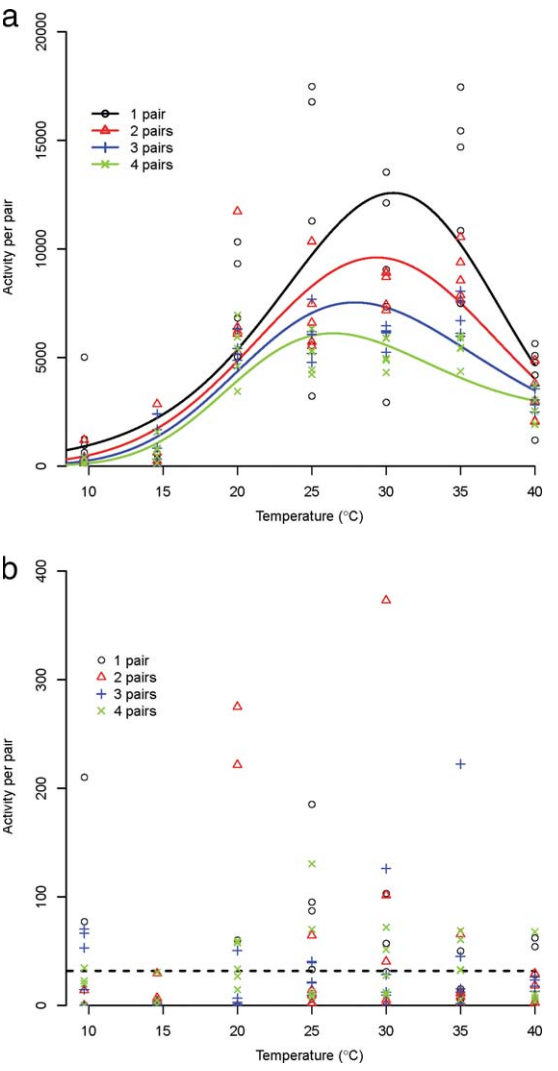

Fig. 1. The effect of density (sex ratio 1:1) and temperature on locomotor activity. Activity of *Musca domestica* measured during (a) daytime and (b) nighttime as total number of times the flies crossed the infrared light beam in the LAM. Activity calculated as the mean number of crossings per pair of flies ($N = 5$). (Figure in color online only.)

Results

Daytime and Nighttime Activity. Locomotor activity varied markedly between daytime and nighttime (Figs. 1 and 2; Supp. Fig. 1[online only]), with the activity several magnitudes higher during daytime. In all cases, daytime and nighttime activity were analyzed separately.

The Effect of Density and Temperature on Activity. Activity varied markedly between the density and temperature treatments (Fig. 1). During daytime density, temperature as well as a nonlinear temperature

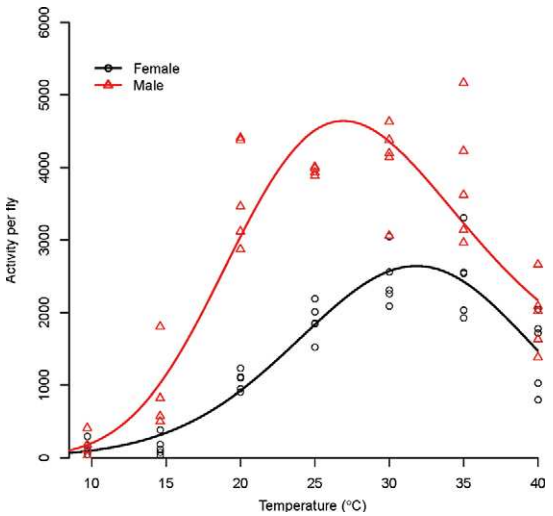

Fig. 2. The effect of sex and temperature on locomotor activity. Mean locomotor activity of *M. domestica* during daytime for each sex, measured as total number of times the flies crossed the infrared light beam in the LAM. Activity calculated per fly at the different temperatures ($N = 5$). (Figure in color online only.)

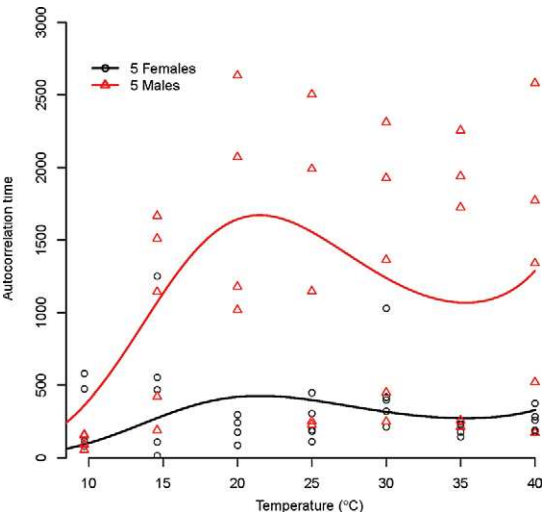

Fig. 3. Predictability of locomotor activity. Autocorrelation time of daytime locomotor activity of *M. domestica*, males and females, under different temperatures. One unit on the y-axis equals to 5 s ($N = 5$). (Figure in color online only.)

term and the interactions between density and the linear and nonlinear temperature terms were significant (Table 1; Fig. 1a). When performing individual analyses at each temperature, density significantly reduced activity at all temperatures tested, which is indicated by negative slopes, except at 15°C during daytime, which had a positive slope (Supp. Table 1 [online only]). The effect of density was highest at 35°C (Supp. Table 1 [online only]). No significant effect of density or temperature was found at nighttime (Table 1; Fig. 1b).

The Effect of Sex and Temperature on Activity. Locomotor activity varied markedly between sexes during daytime, which again was most pronounced at intermediate temperatures. Both sex and temperature and their interaction were highly significant for daytime activity (Table 1; Fig. 2), whereas this was not the case for nighttime activity, where only temperature had a significant effect (Table 1; Supp. Fig. 1 [online only]). Males were significantly more active than females during daytime, except at 10 and 40°C (Supp. Table 2 [online only]; Fig. 2). Locomotor activity was found to be highest at 30 and 35°C for males and females, respectively (Supp. Table 2 [online only]).

Predictability of Activity. The correlation between locomotor activity at different time points was calculated for all treatments (Fig. 3; Supp. Fig. 2 [online only]). There were significant interactions between density and both linear and nonlinear temperature

Table 1. GLM analysis of the effect of temperature and density or sex on locomotor activity and autocorrelation time

Source	Activity daytime	Activity nighttime[a]	Autocorrelation time
Density			
Intercept	25,027 (4580)***	237.6 (135.8)	1,741 (1475)
Density	−6,715 (1577)***	21.8 (7.43)**	−917 (613)
Density^2	—	—	−111 (47)*
Temperature	−4,796 (748)***	−42.4 (19.4)**	−352 (224)
Temperature^2	270 (35)***	2.17 (0.86)*	19.0 (10.1)
Temperature^3	−4 (0)***	−3.2 by 10^{-2}**	−0.29 (0.14)*
Density × temperature	1,162 (261)***	—	247 (84)**
Density × temperature^2	−59 (12)***	—	−11.7 (3.8)**
Density × temperature^3	—	—	0.16 (0.05)**
Sex			
Intercept	17,000 (2844)***	65 (11)***	272 (380)
Sex	−16,890 (2,130)***	—	−2165 (596)***
Temperature	−3,234 (461)***	—	5.6 (33.6)
Temperature^2	187 (22.4)***	—	−0.15 (0.66)
Temperature^3	−2.81 (0.32)***	—	—
Sex × temperature	2,146 (251)***	—	248 (62)***
Sex × temperature^2	−42 (5.4)***	—	−4.34 (1.31)**

[a] Analysis could not converge unless starting values were specified. Therefore, we cannot know for certain if the given values are local or a global optimum. The estimated effects are given with the SE in parentheses. The P values are calculated using Wald's tests.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 2. GLM analysis of the difference between autocorrelation time of each sex at each temperature

Source	Males	Females	Male–female difference
Daytime 10°C	110 (51)	266 (79)	−156 (94)
Daytime 15°C	986 (303)	477 (211)	509 (370)
Daytime 20°C	2,088 (346)	177 (101)	1,911 (360)***
Daytime 25°C	1,245 (336)	247 (151)	978 (368)**
Daytime 30°C	1,260 (329)	474 (202)	786 (386)*
Daytime 35°C	1,279 (309)	205 (124)	1,074 (333)***
Daytime 40°C	1,277 (311)	256 (139)	1,021 (341)**

The estimated effects are given with the SEs as calculated with Wald's tests in parentheses.

The *P* values are calculated with likelihood ratio tests comparing models with and without an effect of sex.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

terms ($P < 0.001$) for the autocorrelation time (Table 1). The combined effects of density and temperature suggested maximum predictability at the intermediate densities and a lower predictability at the high and low temperatures (Table 1; Supp. Fig. 2 [online only]). However, sex and the interaction between sex and temperature had a highly significant effect on the autocorrelation time (Table 1). When looking at the autocorrelation time at the different temperatures tested, the autocorrelation time of males was significantly different from that of females at all temperatures above 15°C (Table 2). At all temperatures, the autocorrelation time of females was virtually constant (Fig. 3). The autocorrelation time of the males was highest at 20°C and decreased at temperatures above (Table 2).

Discussion

Only a limited number of studies have addressed the effect of both temperature and density on the activity of insects. However, it has been shown that both factors can affect feeding activity in *Stomoxys calcitrans* (L.) (Lysyk 1995). The current study demonstrates that temperature indeed has a large impact on the locomotor activity of *M. domestica* during daytime. Locomotor activity increased with temperature in the range from 10 to 30–35°C and decreased when the temperature was above 35°C. Earlier studies have shown that *M. domestica* prefers temperatures around 30°C (Dakshinamurty 1948), which was also the temperature that induced the highest activity in our experiment. Other studies have looked at physical activity at temperatures ranging from 16 to 28°C and found similar trends (Buchan and Sohal 1981). The results furthermore show that males are more active than females at all temperatures, except at 10 and 40°C. The reduced activity at high temperatures is likely to be because of the animals being stressed, as the temperature is above the optimum temperature for *M. domestica.* Stressful temperatures can increase the expression of heat shock proteins in *M. domestica* (Tiwari et al. 1997) and also other energy-expensive physiological responses, which can therefore not be allocated to sustaining behavioral processes. The temperatures chosen in the current study are likely to reflect what flies can be exposed to in and around cattle, chicken, and pig farms. However, it would be interesting in future studies to monitor activity under fluctuating temperatures, as this has been shown to affect thermal performance (Sarup and Loeschcke 2010).

The high daytime activity of males observed could be because of males searching for mating partners, as males are more aggressive in their mating behavior, given that their fitness is optimized by multiple matings (Ragland and Sohal 1973), whereas females most often only mate once and therefore do not search for mating partners when they have been mated (Riemann et al. 1967). The autocorrelation times for females were almost constant across all temperature treatments, which mean that the predictability, or a lack thereof, of female activity was the same for all temperature treatments. Male autocorrelation time and hence predictability increased with temperature up to 25–30°C and then decreased. Male activity was thus more predictable at the intermediate temperatures close to the optimum temperature, where most behavioral processes are likely to occur (Bahrndorff et al. 2012). The lower predictability of locomotor activity of females compared with the males could potentially be linked to mating avoidance behavior of females. However, the evolutionary and ecological consequences of the difference in autocorrelation time between sexes need to be tested experimentally.

In the current study, density reduced daytime activity, but the effect of density decreased at low and high temperatures, compared with the effect at intermediate temperatures. The reduced activity with increased density has also been found in *Drosophila melanogaster* Meigen (Sewell 1979). An explanation for this density-dependent effect of temperature on locomotor activity could be due to the flies being stressed at extreme temperatures, where flies cannot allocate energy into sustaining activity, whereas at intermediate temperatures, behavioral aspects such as courtship behavior (Goulson et al. 1999a) and differences in activity between sexes (Bahrndorff et al. 2012) could explain differences between treatments. The lack of effect of density on fly activity at nighttime was expected because of the low level of activity of flies generally observed during nighttime. *M. domestica* can reach high densities on farm animals, and results from Danish pig and cattle farms show that fly numbers regularly reach 300 flies per animal, but can go much higher (Skovgard and Jespersen 2000). The density treatments used therefore reflect relevant ecological conditions. The results on density could be affected by multiple simultaneous crossings of the infrared light beam. The likelihood of this will in theory increase with density and could potentially deflate the activity estimates at high densities. We consider multiple crossings although as a rare event and the effect of this negligible.

The results of the current study show that the locomotor activity during daytime is highly dependent on both temperature and density. However, further studies are needed to clarify how results translate into

field conditions. Laboratory populations can be exposed to both laboratory selection (Hoffmann et al. 2001) and genetic drift when kept at low population sizes (Briscoe et al. 1992). The population used in the current study was kept at high population sizes (2–3,000), making it unlikely to be severely affected by genetic drift. However, it cannot be ruled out that laboratory selection affected our results. Nevertheless, field studies do indeed suggest that the behavior and dispersal of flies are strongly affected by temperature, and limited dispersal has been observed at extreme low or high temperatures (Loeschcke and Hoffmann 2007, Kristensen et al. 2008). More importantly, it has been shown that locomotor activity is positively correlated with the ability to find resources under field conditions (Overgaard et al. 2010). In addition to field experiments, comparative studies including multiple populations originating from different habitats or the use of isofemale lines could be used to test the evolutionary significance of locomotor activity in future studies (Bahrndorff et al. 2006, 2010).

Several studies have shown how the activity of vectors can affect disease transmission. Parasite infections can lead to changes in host behavior and transmission rate (for review see Schaub (2006)). However, there is a lack of studies addressing the effect of temperature and density on the behavior of insect vectors and/or carriers of pathogens and zoonotic agents. We observed a strong association between temperature, density, and activity. The observed changes in activity may thus result in increased dispersal and/or transmission of pathogens with increased temperatures in *M. domestica.* Models on disease transmissions in social ants have shown how important worker activity is for the spread of disease in ant colonies (Pie et al. 2004). Furthermore, results and dispersal models in grasshoppers suggest that temperature might simply lead to increased activity and dispersal (Walters et al. 2006). Conversely, the predicted increase in population size as a function of climate change (Goulson et al. 2005) could result in habitats with increased density levels, which could reduce the activity of the individual fly. Increased levels of density may thus reduce the effect of increased temperature on activity. However, high density is also known to trigger migration (Sasaki et al. 2000, Feder et al. 2010) and therefore dispersal may consequently increase.

Altogether, the current study showed a significant interaction of temperature and density on daytime locomotor activity in *M. domestica.* Furthermore, locomotor activity was temperature-dependent and changing temperatures may therefore increase locomotor activity and dispersal of houseflies.

Acknowledgments

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